

VALIDATION OF A NEW STANDARDIZED CYSTATIN C TURBIDIMETRIC ASSAY: Evaluation of the CKD-EPI equations in hypertensive patients

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INTRODUCTION

Due to the recently standardization of cystatin C assays using ERM®-DA471/IFCC reference material, there is a growing interest in cystatin C, solely or in combination with creatinine, as an alternative marker of renal function.

AIMS OF THE STUDY

- 1) To evaluate the analytical performances of a new standardized turbidimetric cystatin C assay using Diasys reagents (DiaSys Diagnostic Systems GmbH, Germany) on Olympus AU2700® analyzer.
- 2) To assess clinical relevance by comparison of estimated GFR (eGFR) equations with the GFR measured (mGFR) by a reference isotope method (^{99m}Tc-DTPA) in a population of hypertensive patients.

PATIENTS AND METHODS

ANALYTICAL PERFORMANCES

- Repetability (n=20) and reproductibility (n=30) on three plasma pools (1.1, 1.9 and 4.8 mg/L)
- Linearity according to EP6-A CLSI protocol
- Limit of detection (LoD) according to EP17-A CLSI protocol and limit of quantification (LoQ)

METHOD COMPARISON

- A panel of five serum pools ("Biology of renal function and renal failure" working group (SFBC/SN/SFD))
- Comparison with results generated by the standardized Siemens-PENIA method

CLINICAL EVALUATION

- 100 hypertensive patients (55 male, mean age of 52.9 ± 15.9 years, mean mGFR=90.0 ± 29.7 mL/min/1.73m²)
- Estimation of GFR :
 - IDMS traceable creatinine-based equations : MDRD and CKD-EPI
 - standardized cystatin C-based GFR formulas recently published by the CKD-EPI using cystatin C alone (CKD-EPI cystatin C) and cystatin C in combination with creatinine (CKD-EPI creatinine-cystatin C) (*Inker et al, NEJM 2012*)
- Analysis of bias, precision and accuracy (P20%)

RESULTS

1) ANALYTICAL PERFORMANCES

- All CVs for plasma pools were below 3%
- LoD = 0.10mg/L and LoQ = 0.41 mg/L (CV20%)
- Deviation from linearity at each measured level was less than 3% for the calibrator whereas deviation from linearity could reach up to 10% using patient sample

2) PANEL MEASUREMENT

- The pool analysis showed that standardized Diasys-PETIA assay on AU2700® is closed to the standardized Siemens-PENIA method with a small constant bias of 0.01 mg/L.

3) IMPACT ON GFR ESTIMATION

- In overall population, bias and accuracy of the three CKD-EPI equations were better than for the simplified 175 MDRD. Importantly, the use of the CKD-EPI creatinine-cystatin C equation significantly improved precision and accuracy as compared with the CKD-EPI creatinine equation.
- When considering only the subjects with mGFR >60 mL/min/1.73m² (84% of our population), the results remained the same (Table 1).

Variables	mGFR levels (mL/min/1.73m ²)			
	<60 (n=16) [*]	>60 (n=83)	60-89 (n=29)	≥90 (n=54)
Bias – median difference (95% CI)				
MDRD	-1.9 (-12.5 to 3.7)	-12.7 (-15.9 to -7.4)	-8.1 (-13.0 to -3.3)	-15.5 (-21.3 to -7.8)
CKD-EPI Creatinine	-1.8 (-12.5 to 5.0)	-7.8 (-10.58 to -4.8) ^a	-4.7 (-8.6 to 1.0) ^a	-9.7 (-17.9 to -5.3) ^a
CKD-EPI Cystatin C	-4.7 (-8.4 to 1.1)	-8.2 (-9.9 to -4.6) ^a	-8.4 (-10.8 to -3.0)	-6.4 (-11.4 to -3.2) ^a
CKD-EPI Creatinine-Cystatin C	-4.9 (-10.1 to 0.1)	-7.4 (-10 to -5.6) ^a	-6.0 (-9.1 to -1.9)	-8.0 (-11.3 to -6.0) ^{a,b}
Precision – IQR of the difference 95% CI				
MDRD	16.6 (5.8 to 21.7)	20.2 (14.5 to 27.1)	12.2 (7.3 to 17.5)	27.1 (18.0 to 31.9)
CKD-EPI Creatinine	17.6 (7.9 to 22.6)	20.0 (13.9 to 24.7)	16.3 (7.6 to 23.8)	20.8 (14.1 to 25.2)
CKD-EPI Cystatin C	9.7 (4.5 to 16.1)	15.1 (11.3 to 18.2)	13.7 (5.3 to 20.7)	16.1 (10.7 to 19.6) ^a
CKD-EPI Creatinine-Cystatin C	9.2 (4.2 to 15.4)	11.1 (7.7 to 16.4) ^{a,b}	11.0 (5.2 to 16.4)	10.1 (7.2 to 17.6) ^{a,b,c}
Accuracy – P20 (95% CI)				
MDRD	0.44 (0.19 to 0.69)	0.64 (0.54 to 0.73)	0.66 (0.48 to 0.83)	0.63 (0.50 to 0.76)
CKD-EPI Creatinine	0.50 (0.25 to 0.75)	0.80 (0.70 to 0.88) ^a	0.76 (0.59 to 0.90)	0.81 (0.70 to 0.91) ^a
CKD-EPI Cystatin C	0.50 (0.25 to 0.75)	0.86 (0.77 to 0.93) ^a	0.79 (0.62 to 0.93)	0.89 (0.80 to 0.96) ^a
CKD-EPI Creatinine-Cystatin C	0.69 (0.44 to 0.94)	0.93 (0.87 to 0.98) ^{a,b,c}	0.90 (0.79 to 1) ^a	0.94 (0.87 to 1.00) ^{a,b}

Table 1 : Bias, precision and accuracy (P20) of MDRD, CKD-EPI creatinine (2009), CKD-EPI cystatin C (2012) and CKD-EPI creatinine-cystatin C (2012) equations patients according to different mGFR levels.

^ap<0.05 versus MDRD equation; ^bp<0.05 versus CKD-EPI Creatinine equation, ^cp<0.05 versus CKD-EPI Cystatin C equation (Mc Nemar Test).

*No statistical analysis was performed in patients mGFR<60mL/min/1.73m² because of insufficient sample size (n=16).

CONCLUSION

Cystatin C measurements using Diasys reagents have reliable analytical performances.

Combining standardized creatinine and standardized cystatin C in the same equation could produce a more precise estimation of renal function in the reference range.

